ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1998, p. 263–268 Vol. 42, No. 2
0066-4804/98/$04.00+0

Toxicological Profile and Pharmacokinetics of a Unilamellar Liposomal Vesicle Formulation of Amphotericin B in Rats

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Received 9 May 1997/Returned for modification 10 October 1997/Accepted 15 November 1997

AmBisome (ABLP) is a unilamellar liposomal preparation of amphotericin B that has demonstrated an improved safety profile compared to conventional amphotericin B. Single- and multiple-dose pharmacokinetics were determined by using noncompartmental methods for rats administered ABLP at 1, 3, 9, and 20 mg/kg/day. The toxicological profile was evaluated following 30 consecutive days of intravenous ABLP administration. Mean plasma amphotericin B concentrations reached 500 and 380 μg/ml (males and females, respectively) following 30 days of ABLP administration at 20 mg/kg. The overall apparent half-life was 11.2 ± 4.5 h (males) or 8.7 ± 2.2 h (females), and the overall clearance (CL) was 9.4 ± 5.5 ml/h/kg (males) or 10.2 ± 4.1 ml/h/kg (females). ABLP appears to undergo saturable disposition, resulting in a non-dose-proportional amphotericin B area under the curve and a lower CL at higher doses. Histopathological examination revealed dose-related transitional-cell hyperplasia in the transitional epithelium of the urinary tract (kidney, ureters, and urinary bladder) and moderate hepatocellular necrosis at the 20-mg/kg/day dose. Administration of ABLP in doses of up to 20 mg/kg/day resulted in 100-fold higher plasma amphotericin B concentrations, with significantly lower toxicity than that reported with conventional amphotericin B therapy.

Amphotericin B is a potent intravenous antifungal drug with demonstrated efficacy against many important pathogenic mycoses (1). A highly lipophilic and practically insoluble compound, amphotericin B is most commonly administered as a deoxycholate micellular dispersion (DAMB). The use of amphotericin B is limited by acute and chronic toxicities, including headache, chills, fever, nausea, vomiting, hypokalemia, and anemia, with dose-dependent nephrotoxicity occurring with prolonged therapy in 80% of patients (3). In many cases, the dose and duration of amphotericin B use are limited by toxicity rather than by the clinical status of the patient.

Lipid complex formulations of amphotericin B have demonstrated a reduced toxicity profile relative to and associated with the administration of DAMB. Decreased renal tubular toxicity was demonstrated in rats given liposomal amphotericin B at 1.5 or 3.5 mg/kg compared to the same dose of amphotericin B in dimethyl sulfoxide–phosphate-buffered saline (13). In mice, the 50% lethal dose of DAMB was 1.2 mg/kg, whereas minimal toxicity was found with a liposomal amphotericin B dose of 12 mg/kg (14). Studies have demonstrated that the disposition and toxicity of liposomal amphotericin B is influenced by both the size and composition of the liposome (5, 8). Small (<0.15-μm), unilamellar liposomes are taken up more slowly by the reticuloendothelial system (RES) and have a longer apparent circulating half life in plasma. Larger liposomes and multilamellar liposomes are rapidly removed from the circulation by phagocytosis and localize in the liver and spleen, limiting their potential systemic exposure.

ABLP is a unilamellar liposomal preparation of amphotericin B that is less than 100 nm in diameter (1). The administration of this unilamellar formulation to rats resulted in the death of 1 of 10, 5 of 10, and 9 of 10 animals as doses of 25, 50, and 75 mg/kg/day; respectively, for 30 days. In the surviving animals, there was a marginal elevation of blood urea nitrogen (BUN), whereas elevated asparate aminotransferase (AST) was only found at 50 and 75 mg/kg/day (16). The current study was undertaken to obtain a toxicological profile of ABLP in rats at clinically relevant doses and to determine the pharmacokinetic parameters of amphotericin B when administered as ABLP.

MATERIALS AND METHODS

Animals. Sprague-Dawley Cr1:CD (SD) BR rats (214 males and 215 females), approximately 42 days old and weighing 175 to 237 g (males) or 143 to 182 g (females) on the day prior to study initiation, were used. The animals were housed in individual stainless steel cages in a temperature-, humidity-, and light-controlled room and were allowed free access to food and water throughout the study, except for overnight fasts prior to clinical pathology blood sampling. Males and females were housed in separate rooms. Animals were acclimatized to the laboratory environment for 2 weeks prior to study initiation. The study was conducted in an American Association for Accreditation of Laboratory Animal Care-accredited testing facility under a protocol that was approved by their Animal Use Committee.

Test material. A ABLP (AmBisome; NeXstar, San Dimas, Calif.) was used. Each vial contained 50 mg of amphotericin B, sucrose, phosphatidylcholine, distearoylphosphatidylglycerol, cholesterol, disodium succinate, and α-tocopherol. ABLP vials were stored under refrigeration (4°C) and protected from light. Prior to use, the lyophilized ABLP was reconstituted with sterile water and diluted within 12 h of administration to concentrations of 0.1, 0.6, 1.8, and 4 mg of amphotericin B per ml with a 5% dextrose solution to provide a constant 5-ml/kg dose volume. The sterile 5% dextrose solution and a 0.9% sodium chloride solution were purchased from commercial sources. Control animals were administered either a nondrug liposome formulation (NeXstar) or a 5% dextrose solution at a dose volume of 5 ml/kg.

Study design. Animals were randomized into six groups (13/sex/group) to provide the 30-day toxicologic and pharmacokinetic profile (groups 1 to 6). Control animals received a 5% dextrose solution (group 1) or a nondrug, liposome-containing preparation (group 2) for a period of 30 days. The multiple-dose animals (groups 3 to 6) received ABLP infusions of these same doses (respectively) daily for 30 days. An additional four groups of 18 animals/sex/group provided a single-dose pharmacokinetic evaluation (groups 7 to 10). Additional animals were included in groups 5, 7, 8, 9, and 10 (3/sex/group) and in group 6 (10/sex) to ensure an adequate number of evaluable animals at study termination in the event of early death or moribund-animal sacrifice. Groups 7 to 10 received a single intravenous dose of ABLP (1, 3, 9, or 20 mg/kg, respectively) over 1 min via a tail vein.

Blood samples were collected from three animals per sex per group at 1, 3, 5, 8, and 24 h following the first (single-dose groups) or the last (multiple-dose

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groups) AmBisome dose. Terminal blood samples (approximately 4.5 ml) for amphotericin B concentration analysis were collected (under sodium pentobarbital anesthesia) from the vena cava into sodium citrate Vacutainer tubes. An additional blood sample was collected at 0.5 h postadministration of the drug from the animals in the single-dose groups. The plasma was separated by centrifugation, and approximately 1 ml was frozen at −15°C until assayed for total amphotericin B concentrations. When possible, the remaining plasma from animals in groups 6 and 10 was retained for ultracentrifugation to obtain a protein-free fraction, which was then frozen until assayed for nonliposomal, non-protein-bound amphotericin B. Tissue samples were collected from three animals per sex per group at the same time as the blood samples and stored frozen until assayed for total amphotericin B concentrations.

Pathology. Animals were observed twice daily for signs of toxicity. Body weights were recorded the day before dosing began, daily on each dosing day, and immediately prior to sacrifice. Food consumption was recorded weekly during the dosing period. Blood samples for laboratory evaluation were obtained from the retroorbital plexus on days 8, 15, and 28 and, when possible, from moribund animals (from the abdominal aorta). Necropsies were performed on all surviving animals at termination and on the moribund animals sacrificed. At terminal sacrifice, brain, kidney, liver, lung, and spleen tissue samples were collected for assay of amphotericin B concentrations in tissue. Tissue samples were blotted dry, weighed, frozen in liquid nitrogen, and then stored frozen (−80°C) until amphotericin B concentrations were assayed. Representative samples of these organs and additional selected tissues were collected, processed, and stored in 10% phosphate-buffered formalin for histopathological examination. Processed tissue sections were stained with hematoxylin and eosin and examined microscopically. Tissues from moribund animals were similarly collected and processed, except that samples for drug analysis were stored directly at −80°C, as liquid nitrogen was not available.

High-pressure liquid chromatography assay. Samples (100 μl) of rat plasma, protein-free rat plasma filtrate, analytical standards, or quality controls and 300 μl of methanol were mixed well in a polyethylene tube, which was then placed in a 50°C water bath for 15 min. The tube was cooled at room temperature for 5 min and then centrifuged for 10 min at 15,000 rpm (9,500 × *g*). The supernatant was placed in an injector vial, and 50 μl was injected onto a Hypersil octyldecyl silane 5-μm particle size analytical column (150 by 4.6 mm [inside diameter]) (2). Detection was accomplished with a UV–visible-light detector set at 382 nm. The mobile phase consisted of methanol–1 mM disodium EDTA containing 82 mM triethylamine and 96 mM phosphoric acid–deionized water (8:1:1.25, vol/vol/vol) delivered at a flow rate of 0.8 ml/min. The linear range of the assay was 50 to 50,000 ng/ml (correlation coefficients, >0.996), and the limit of quantitation (LOQ) was 50 ng/ml, with a relative standard deviation (RSD) of 8.0%. The absolute recovery was 95.4%. Concentrations of amphotericin B in plasma were calculated by using weighted (1/concentration) least-squares linear regression of amphotericin B peak height and external-standard quantitation with an interday relative standard deviation (SD) of 4.6 to 10.4%.

For tissue samples, weighed aliquots (approximately 0.5 g) of either blank rat tissue (same type as the sample) from rats not receiving ABLP or sample tissue were placed in a glass tube along with 4.5 ml of methanol and 0.5 ml of 10 mM phosphate buffer (pH 7.4). The samples were homogenized for 5 s with a tissue homogenizer, mixed on a vortex mixer for 5 min, and then centrifuged until the supernatant was clear. A 300-μl aliquot of the supernatant was place into an autoinjector vial, and 120 μl was injected into a C_{18} 10-μm particle size analytical column (216 by 4.6 mm [inside diameter]) (Whatman Inc., Clifton, N.J.) maintained at 35.5°C. The mobile phase consisted of acetonitrile–10 mM sodium acetate (39.4:60.6, vol/vol) delivered at a flow rate of 1 ml/min. Amphotericin B was detected with a UV–visible-light detector set at 382 nm. Concentrations of amphotericin B in tissue were calculated by using a natural-logarithm transformation of a quadratic regression equation ($\ln \text{peak area} = \ln A + B \cdot \ln[\text{concentration}] + C \cdot \ln[\text{concentration}]^2$) of amphotericin B peak areas and external standard quantitation. The assay linear range was 0.50 to 500.0 μg/g (correlation coefficients, >0.9950) with an LOQ of 0.50 μg/g (RSD, 6.8%). Assay of quality control samples of 0.50 to 500.0 μg/g yielded recoveries of 100.3% (0.50 μg) and 100.7% (500 μg) with RSDs of 6.8 and 2.9%, respectively.

Data analysis. Descriptive statistics for toxicology variables (i.e., body weight gain, food consumption, organ weight, clinical pathology values, and organ-to-body weight percentages and ratios) were calculated. Homogenicity of the variance of data for each variable was assessed by using Bartlett's test with a significance levels of 0.001. Significance levels of 0.05, 0.01, and 0.001 (two sided) were used for all other statistical assessments. For variables with homogeneous variance, an analysis of variance, followed by Dunnett's test, was conducted. For variables with heterogeneous variance, the Kruskall-Wallis test and the Wilcoxon rank test were performed.

The data for the mean amphotericin B concentration in plasma versus time was analyzed by noncompartmental methods. The area under the plasma-versus-time curve (AUC) and the terminal-phase elimination rate constant (β) were calculated by using the nonlinear least-squares curve-fitting program RStrip (15). The AUC from time zero to 24 h (AUC_{0-24}) was calculated by using the linear trapezoidal method for both plasma and tissue samples. The terminal elimination half-life (apparent $t_{1/2}$) was calculated as 0.693/β, where β is the negative slope (derived by unweighted least-squares regression of the final three concentration-time points) of the natural log-linear terminal portion of the plasma concentration-versus-time curve. The total AUC ($AUC_{0-\infty}$) was obtained as the sum of $AUC_{0-24} + C_{last}/\beta$, where C_{last} is the last observed concentration. Total body clearance (CL) was calculated from $CL = \text{dose}/AUC_{0-\infty}$; the volume of distribution (V) was calculated from $V = CL/\beta$. When performed, statistical comparisons of pharmacokinetic parameters were conducted by using Student's *t* test.

RESULTS

Observations and necropsy. After two doses, there were 12 female mortalities at 20 mg/kg/day (five deaths and seven moribund-animal sacrifices). These deaths were considered to be related to ABLP treatment; there were no other treatment-related deaths during the duration of the study. Other deaths, one male each at 1, 3, and 20 mg/kg/day, were not considered treatment related. Clinical signs present in females that died or were sacrificed included red/orange vaginal discharge, coldness to touch, hunched posture, partly closed eyes, reduced activity, and clonic convulsions (in one animal only). One female in the 20-mg/kg/day group exhibited these symptoms, but they subsided after the second dose and the animal survived the 30-day dosing regimen. Other clinical signs occurring in a few animals at 20 mg/kg/day only included brown abdominal/urogenital staining, ataxia, lying on the side, and dehydration. No consistent drug-related observations about any other animals were made.

No drug-related effects were noted in either sex in the liposome controls or the 1- or 3-mg/kg/day group compared to the dextrose controls. The overall weight gain of males given 9 mg/kg/day was low and was significantly lower than that of dextrose controls ($P < 0.01$) during the last 2 weeks of the study. Males in the 20-mg/kg/day group had significantly lower weight gains (mean ± SD) than did dextrose controls ($P < 0.01$) throughout the dosing period (88 ± 21 versus 141 ± 24 g, respectively). No significant difference in weight gain was seen in females given 9 mg/kg/day compared to dextrose controls. Females in the 20-mg/kg/day group showed variable weight gains compared to dextrose controls, but the overall body weight gain over the entire dosing period was significantly ($P < 0.01$) lower than for these controls (40 ± 9.5 versus 54 ± 15 g, respectively). Food consumption by both sexes in the high-dose group was reduced consistent with body weight loss. No effect on food consumption was noted for animals in any of the remaining dose groups (either sex) compared to the dextrose controls.

At termination, gross pathology findings (occurring with an increased incidence in males and females administered ABLP at 9 or 20 mg/kg/day) included ureter dilatation or thickening, urinary bladder dilatation, enlarged lymph nodes and spleen, and discoloration and presence of pale areas or foci in the liver. Group mean kidney weight relative to body weight (grams per 100 g of body weight) was increased in males and females (0.44 to 0.45 and 0.48 to 0.52, respectively) at both 9 and 20 mg/kg/day compared to either dextrose controls (0.35 and 0.39 for males and females, respectively) or liposome controls (0.35 and 0.37 for males and females, respectively). Mean kidney weight in the 1-mg/kg/day males was increased compared to that of dextrose controls ($P < 0.001$), but this effect was not observed in females. Group mean kidney weight compared to body weight for females in all ABLP dose groups was significantly increased ($P < 0.001$) compared to that of liposome controls.

Microscopic pathology. Histopathological evaluation of those females in the 20-mg/kg/day group which died or were moribund sacrifices revealed moderate-to-severe hepatocellular necrosis of the liver to be the primary lesion. This was considered to be the most likely cause of the poor condition

TABLE 1. Summary of key clinical chemistry and hematology findings at study termination

Dose (mg/kg/day)	Mean values for males/females ± SD[a]			
	Platelet count (10^3)	BUN (mg/dl)	AST (U/liter)	ALT (U/liter)
0[b]	1,064 ± 91.3/1,117 ± 153.9	14.1 ± 1.4/18.5 ± 2.2	120.5 ± 13.9/132.3 ± 30.2	37.0 ± 3.6/37.5 ± 3.9
0[c]	1,029 ± 111.1/1,071 ± 83.2	15.1 ± 1.7/18.0 ± 2.9	126.9 ± 23.3/128.1 ± 26.7	38.4 ± 4.0/37.3 ± 6.0
1	1,050 ± 115.6/1,053 ± 99.5	17.2 ± 2.0/21.0 ± 4.0	107.5 ± 22.9/104.1 ± 18.6	35.9 ± 6.2/35.1 ± 5.1
3	982.5 ± 51.4/1,016 ± 57.6	25.5 ± 4.1/23.3 ± 3.5	94.8 ± 10.8/107.9 ± 15.9	31.9 ± 4.6/30.4 ± 4.7
9	879.3 ± 78.4/940.8 ± 107.0	47.7 ± 7.9/51.8 ± 10.8	105.5 ± 14.1/236.9 ± 172.2	31.6 ± 5.0/95.6 ± 89.0
20	792.6 ± 137.0/724.1 ± 75.0	60.2 ± 7.4/71.4 ± 8.7	100.3 ± 16.4/154.8 ± 44.5	27.9 ± 5.1/47.2 ± 13.6

[a] Reference ranges: platelet count (10^3), 753 to 1,447 (males) and 786 to 1,508 (females); BUN (milligrams per deciliter), 9.3 to 24.8 (males) and 9.6 to 31.4 (females); AST (units per liter), 80 to 206 (males) and 86 to 222 (females); ALT (units per liter), 26 to 27 (males) and 22 to 54 (females).
[b] Dextrose controls.
[c] Liposome controls.

and death of these animals. Microscopically, a change described as "foamy-cell accumulation" was observed in many organs of animals of both sexes that received ABLP and in the kidneys of liposome controls. These foamy cells were suspected to be macrophages. This change was noted in the livers of all groups (male or female) administered ABLP with an apparent dose-related increase in severity. Transitional-cell hyperplasia was observed in the transitional epithelium of the urinary tract (kidneys, ureters, and urinary bladder) of males and females in all ABLP treatment groups, and the severity was dose related. In the kidneys and ureters of some animals, this hyperplasia was characterized by a thickened, often basophilic epithelium with occasional mitotic figures accompanied by a mixed-cell infiltration (mainly neutrophils).

Clinical chemistry and hematology. Key clinical chemistry and hematologic findings at study termination are given in Table 1. No clinically or statistically significant hematologic profile changes were observed in animals administered ABLP at 1 or 3 mg/kg/day or in the liposome control group. A dose-related and statistically significant ($P < 0.05$) decrease in the group mean platelet count was observed for males and females administered ABLP at 9 and 20 mg/kg/day compared to dextrose or liposome controls. Although this decrease was significant relative to controls, in general, the mean values were not below the normal laboratory range. No other toxicologically significant changes in associated hematology parameters were found in these animals.

There was a significant dose-related increase ($P < 0.01$) in BUN at 3, 9, and 20 mg/kg/day in both sexes compared to either the respective dextrose or liposome control groups. However, only the group mean BUN at 9 and 20 mg/kg/day (male and female) was elevated outside of the normal laboratory range. At 20 mg/kg/day, increased mean alanine aminotransferase (ALT) activity was found in males compared to males in the dextrose and liposome control groups, but the value was within the normal laboratory range. Females in the 9- and 20-mg/kg/day groups showed increases ($P < 0.05$) in serum AST, serum ALT, and alkaline phosphatase activities compared to the dextrose and liposome control groups. There were no other changes in clinical chemistry parameters of toxicological significance considered to be related to the administration of ABLP or liposomal controls.

Plasma pharmacokinetics of amphotericin B. Amphotericin B concentrations in plasma increased disproportionately with increasing doses of ABLP in both males and females following a single intravenous dose and after 30 days of continuous daily administration (Fig. 1 and 2, respectively; Table 2). Similarly, the AUC_{0-24}, when normalized for the dose, was not proportional to the administered dose following either single or multiple administration (Fig. 3). The apparent $t_{1/2}$ (mean ± SD) did not significantly differ between males and females after a single dose (9.2 ± 0.6 and 8.8 ± 3.2 h, respectively; $P = 0.787$) or multiple doses (13.2 ± 6.0 and 8.6 ± 1.0 h, respectively; $P = 0.221$). In addition, the apparent $t_{1/2}$s across doses from the

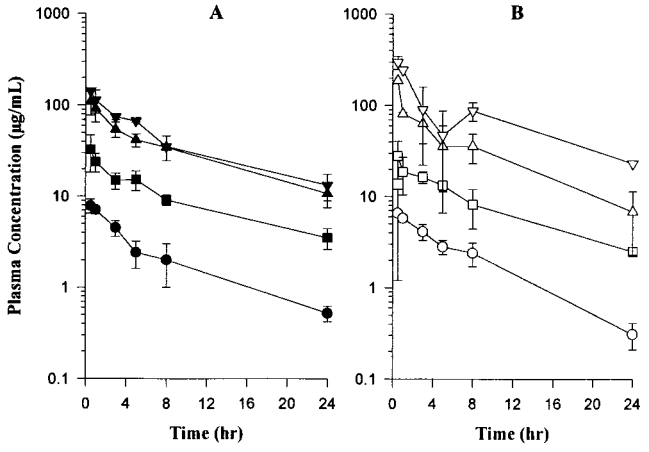

FIG. 1. Mean (± SD) amphotericin B concentrations in the plasma of male (closed symbols) and female (open symbols) rats following administration of a single dose of ABLP of 1 (circles), 3 (squares), 9 (triangles), or 20 (inverted triangles) mg/kg.

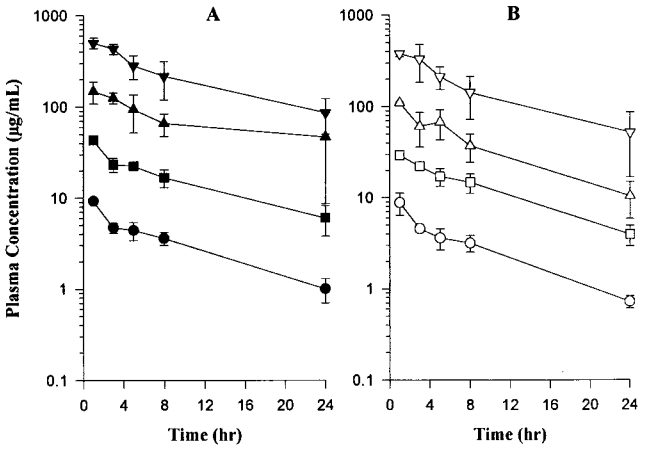

FIG. 2. Mean (± SD) amphotericin B concentrations in the plasma of male (closed symbols) and female (open symbols) rats following administration of 30 consecutive daily doses of ABLP of 1 (circles), 3 (squares), 9 (triangles), or 20 (inverted triangles) mg/kg.

TABLE 2. Amphotericin B concentrations in rat plasma after single and multiple administrations of ABLP[a]

Study day and dose (mg/kg/day)	Sex[b]	Mean concn (μg/ml) ± SD[c] at:					
		0.5 h	1 h	3 h	5 h	8 h	24 h
1							
1	M	7.9 ± 1.4	7.1 ± 0.7	4.5 ± 0.9	2.4 ± 0.8	2.0 ± 1.0	0.52 ± 0.10
	F	6.6 ± 5.4	5.8 ± 0.5	4.1 ± 0.8	2.8 ± 0.5	2.4 ± 0.7	0.31 ± 0.10
3	M	32.6 ± 14.3	23.9 ± 5.6	14.9 ± 3.0	15.2 ± 3.7	9.1 ± 1.2	3.5 ± 0.9
	F	28.0 ± 12.4	18.7 ± 8.3	16.4 ± 2.4	13.3 ± 1.6	8.2 ± 3.8	2.5 ± 0.1
9	M	109.7 ± 31.6	91.5 ± 26.2	54.2 ± 10.6	41.3 ± 6.6	34.3 ± 2.3	10.7 ± 3.2
	F	188.6[d]	81.4 ± 5.5	63.4[d]	35.7 ± 31.5	35.9 ± 10.0	6.9 ± 0.2
20	M	143.0[d]	113.5 ± 32.3	74.6 ± 5.8	66.9 ± 2.6	35.1 ± 10.7	13.2 ± 4.3
	F	297.3 ± 49.0	244.8[d]	90.8 ± 68.5	47.2 ± 40.6	87.4 ± 20.0	23.3[d]
30							
1	M	NS[e]	9.3[c]	4.7 ± 0.6	4.4 ± 1.0	3.6 ± 0.6	1.0 ± 0.3
	F	NS	8.8 ± 2.4	4.6 ± 0.4	3.6 ± 1.0	3.2 ± 0.6	0.7 ± 0.1
3	M	NS	43.2 ± 1.9	24.3 ± 4.2	22.4 ± 2.4	16.7 ± 3.6	6.0 ± 2.2
	F	NS	29.4 ± 3.3	22.1 ± 1.2	17.1 ± 3.8	14.7 ± 3.5	4.0 ± 1.0
9	M	NS	148.0 ± 39.8	125.2 ± 17.2	93.7 ± 42.0	65.6 ± 18.2	46.8 ± 38.2
	F	NS	110.2 ± 3.2	61.0 ± 25.1	67.8 ± 24.4	37.2 ± 12.8	10.5 ± 4.6
20	M	NS	500.4 ± 65.9	431.2 ± 55.3	280.8 ± 81.8	216.2 ± 98.0	86.4 ± 36.5
	F	NS	379.7	331.4	212.6 ± 59.8	142.7 ± 70.6	52.4 ± 35.5

[a] Day 1 data are for the single dose, and day 30 data are for multiple doses.
[b] M, male; F, female.
[c] Unless noted otherwise, $n = 3$.
[d] $n = 2$.
[e] NS, no sample scheduled.

single-dose study were comparable to those in the multiple-dose study (Table 3). The combined day 1 and day 30 apparent $t_{1/2}$s were 11.2 ± 4.5 h in males and 8.7 ± 2.2 h in females. As expected, based on the AUC and apparent $t_{1/2}$ values, the CL tended to decrease as the dose increased following both single and multiple doses (Table 3). The combined day 1 and day 30 CLs of males (9.4 ± 5.5 ml/h/kg) and females (10.2 ± 4.1 ml/h/kg) were comparable.

The protein-free (and presumably liposome-free) filtrates obtained from plasma of animals dosed at 20 mg/kg/day for 1 or 30 days showed no nonliposomal, non-protein-bound amphotericin B. Thus, the unbound amphotericin B (i.e., the free fraction) at the highest dose employed was below the LOQ (0.05 μg/ml) for the analytical methods employed in this study.

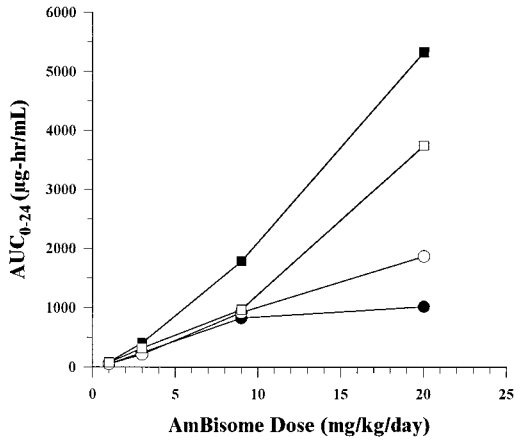

FIG. 3. AUC_{0-24} versus dose following administration of a single dose (circles) or 30 consecutive daily doses (squares) of ABLP to male (closed symbols) or female (open symbols) rats.

No further attempts to measure amphotericin B fractions were made.

Tissue pharmacokinetics of amphotericin B. The highest concentrations of amphotericin B following ABLP administration were present in the liver and spleen. The levels of amphotericin B in the kidneys and lungs were approximately 10 to 20% of those measured in the liver and spleen (Table 4). The drug concentrations in the brains of both males and females given the lower two doses were below the assay LOQ, and only negligible amounts were present at the higher doses. Amphotericin B concentrations in tissue increased linearly with the ABLP dose, except in liver tissue. In females on day 1 and in both males and females on day 30, the amphotericin B concentrations in the livers of the 20-mg/kg/day group were lower than expected (based on the dose), suggesting a possible saturable tissue uptake mechanism.

DISCUSSION

The present study was designed to provide a toxicologic and pharmacokinetic profile of ABLP in rats following 30 days of administration. Nephrotoxicity is the major dose-limiting toxicity of amphotericin B (and colloidal amphotericin B) in rats and has been demonstrated following infusions of DAMB at 1.5 mg/kg (13) and an amphotericin B colloidal dispersion (ABCD) at 5 mg/kg/day (4); comparable data for colloidal amphotericin B (ABLC) has not been published. In the present study, minimal nephrotoxicity was seen with ABLP infusions of up to 20 mg/kg. Relatively small increases in BUN without concomitant rises in serum creatinine concentrations and negligible kidney pathology indicated minimal nephrotoxicity with 30 days of ABLP administration. Elevations in serum ALT, AST, and alkaline phosphatase and liver necrosis clearly demonstrated hepatoxoticity, especially at the two highest doses, and were the most likely cause of drug-related deaths, and females were more sensitive to this effect than were males.

TABLE 3. Amphotericin B pharmacokinetic parameter estimates in rats[a] after single and multiple administrations of ABLP

Study day and dose (mg/kg/day)	Sex[b]	$t_{1/2}$ (h)	MRT[c] (h)	$C_{max,obs}$[d] (μg/ml)	AUC_{0-24} (μg · h/ml)	$AUC_{0-\infty}$ (μg · h/ml)	$AUMC_{0-24}$[e] (μg · h²/ml)	$AUMC_{0-\infty}$[e] (μg · h²/ml)	V (ml/kg)	CL (ml/h/kg)
1										
1	M	8.5	9.3	7.9	53.0	59.4	318.7	549.7	206	16.8
	F	5.7	7.0	6.6	52.4	54.9	306.1	385.7	150	18.2
3	M	9.7	12.3	32.6	242	290	1,691	3,578	145	10.3
	F	8.5	10.3	28.0	212	242	1,402	2,505	152	12.4
9	M	9.7	11.6	109	825	975	5,645	11,309	129	9.2
	F	7.6	7.7	189	918	993	5,033	7,669	99	9.1
20	M	9.0	10.9	143	1,014	1,186	6,675	13,035	219	16.9
	F	13.2	13.8	300	1,861	2,305	12,622	31,778	165	8.7
30										
1	M	8.9	10.9	9.3	82.8	96.0	563.4	1,048	134	10.4
	F	7.9	9.3	8.8	73.5	81.7	465.7	757.2	140	12.2
3	M	10.3	12.9	43.2	405	494	2,913	6,392	90	6.1
	F	8.8	11.1	29.4	320	370	2,269	4,105	103	8.1
9	M	22.1	30.3	148	1,784	3,272	16,108	99,172	88	2.8
	F	7.6	9.4	110	968	1,083	6,223	10,214	91	8.3
20	M	11.5	14.5	500	5,329	6,759	39,868	97,813	49	3.0
	F	9.9	12.2	380	3,742	4,491	26,125	54,797	64	4.5

[a] Based on mean amphotericin B concentrations in plasma. Day 1 data are for the single dose, and day 30 data are for multiple doses.
[b] M, male; F, female.
[c] MRT, mean residence time. $C_{max,obs}$, observed maximum concentration.
[d] $C_{max,obs}$, observed maximum concentration.
[e] AUMC, area under the first-moment curve.

The hepatocellular necrosis and urothelial hyperplasia noted in this study have also been reported in rats given ABCD (4). Similar elevations in AST and ALT values and fatty infiltration of the liver were seen in dogs given ABCD at 5 to 10 mg/kg/day or DAMB at 0.6 mg/kg/day (6). The foamy-cell accumulation seen in the liver, kidneys, spleen, lymph nodes, and adrenals was considered to be an adaptive response to the drug formulation and not a toxicological response. Thus, toxic manifestations seen with ABLP administration were qualitatively similar to those reported with other amphotericin B formulations, although less in magnitude on a equidose basis. In this study, urinary tract hyperplasia was noted at all dose levels, resulting in a no-observable-effect level of less than 1 mg/kg.

The amphotericin B concentrations in plasma were noteworthy. In this study, amphotericin B concentrations in plasma were substantially higher with ABLP administration than those that have been achieved with equivalent doses of either DAMB or ABCD. The mean maximum amphotericin B concentration in the plasma of rats 1 h after administration of a single dose of 1 mg of DAMB or ABCD per kg has been reported as 0.275 or 0.102 μg/ml, respectively (4); after the same ABLP dose in this study, the mean concentrations in plasma 1 h postadministration of the dose were 7.1 and 5.8 μg/ml for males and females, respectively. An increase in the ABCD dose to 5 mg/kg increased the mean 1-h amphotericin B concentration in plasma to 0.170 μg/ml, while an AmBisome dose of 3 mg/kg yielded mean 1-h amphotericin B concentrations of 23.9 and 18.7 μg/ml of plasma in males and females, respectively.

TABLE 4. Mean AUC_{0-24} of amphotericin B in rats administered ABLP intravenously once daily for 30 days

Study day and dose (mg/kg/day)	Mean AUC_{0-24} (μg · h/ml) for males/females			
	Liver	Kidneys	Spleen	Lungs
1				
1	313/275	18/6	126/130	
3	892/628	52/37	274/335	55/30
9	2,288/1,834	174/125	1,149/1,083	224/190
20	3,611/2,681	352/259	2,447/2,691	423/417
30				
1	3,467/3,028	65/54	627/934	35
3	9,818/9,787	350/251	5,220/4,899	2,645/217
9	22,441/22,263	1,549/745	28,122/16,189	1,251/786
20	29,781/29,422	3,278/2,253	49,908/47,102	3,377/2,554

As noted above, the amphotericin B concentrations in plasma and AUCs were not proportional to the ABLP dose, suggesting the presence of saturable disposition processes. Saturable elimination of liposomal amphotericin B from the plasma would be consistent with the known mechanism of liposome clearance by the RES (9, 10). In mice administered ABLP at 5 mg/kg, Proffitt et al. (16) demonstrated that as the levels of amphotericin B in plasma decreased, those in the spleen and liver increased, suggesting RES uptake. Saturable elimination of ABLP has also been reported in rabbits with doses of 0.5 to 10 mg/kg and was thought to be due to saturable uptake by the RES (12). It is interesting that despite a possible saturable disposition of ABLP in rats in other studies, the apparent terminal $t_{1/2}$ in the present study remained relatively constant. The combined male and female apparent $t_{1/2}$s across all doses between days 1 and 30 were 9.0 ± 2.1 and 10.9 ± 4.7 h, respectively. However, this may have resulted from using data which was in the pseudolinear portion of the plasma concentration-time profile to calculate the apparent $t_{1/2}$. Proffitt et al. (16) found a similar apparent $t_{1/2}$ (7.56 h) in female rats administered AmBisome at 5 mg/kg. Amphotericin B CL decreased as the dose increased following single- and multiple-dose ABLP administration, reflecting the disproportionate increase in AUC with the dose.

The distribution of amphotericin B in tissue following ABLP administration was consistent with that previously reported in rats (16), mice (17), and rabbits (12). The highest amphotericin

B concentrations were present in the organs of the RES (spleen and liver), with lesser amounts in the kidneys and lungs and minimal amounts in the brain, supporting the premise that the RES is a major pathway for the elimination of ABLP from the plasma. Concentrations of amphotericin B in tissue on day 30 were substantially higher than on day 1 in all of the tissues sampled, suggesting accumulation of amphotericin B. Francis et al. (7) found a dose-proportional response of pulmonary injury in persistently neutropenic rabbits with experimental pulmonary aspergillosis treated with ABLP at 1, 5, and 10 mg/kg/day. Dose-response relationships of improved antifungal efficacy have also been described by Lopez-Bernstein et al. (14) and Walsh et al. (18). Drug accumulation in tissue with repeated doses may play a significant role in the enhanced efficacy observed with ABLP, since systemic fungal infections are often localized in the liver, lungs, and spleen (14). Other investigators have demonstrated that encapsulation of amphotericin B into liposomes protects the kidneys from the toxic effects of amphotericin B in rats (13) and rabbits (11), which may be the reason for the lack of toxicity seen in the kidneys despite the high concentrations in tissue. During sample analysis, extraction of plasma or tissue samples results in destruction of the ABLP liposome, releasing the encapsulated amphotericin B. Thus, it was not possible to differentiate "free" (nonliposomal) and liposomal amphotericin B.

In the current study, administration of ABLP in doses of up to 20 mg/kg/day was shown to result in a 100-fold higher amphotericin B concentration in plasma than previously seen in rats while demonstrating much less toxicity than that which occurs with conventional DAMB therapy or with ABCD. As previously reported, ABLP appears to undergo saturable disposition involving RES uptake, resulting in non-dose-proportional amphotericin B AUCs and lower CL at doses greater than 1 mg/kg/day. Unlike DAMB, ABLP demonstrated little nephrotoxicity in rats but did show moderate hepatotoxicity, especially at the higher doses administered in the present study.

ACKNOWLEDGMENTS

We thank Ellen Hodosh and Nancy Crorkin for their help in preparing the manuscript.

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